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Short communication

Immune responses and protective efficacy of a recombinant swinepox virus co-expressing HA1 genes of H3N2 and H1N1 swine influenza virus in mice and pigs

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ABSTRACT

The recombinant swine poxvirus rSPV/H3-2A-H1 co-expressing HA1 genes of H3N2 and H1N1 subtype SIV has been constructed and identified. Inoculations of rSPV/H3-2A-H1 yielded ELISA and neutralization antibodies against SIV H1N1 and H3N2, and elicited potent H1N1 and H3N2 SIV-specific INF-γ response from T-lymphocytes in mice and pigs in this study. Complete protection against SIV H1N1 or H3N2 challenge in pigs was observed.

1. Introduction

The gene of RNA segment 4 of influenza A viruses encodes the large hemagglutinin (HA) glycoprotein which is considered the main immune antigen (Hessel et al., 2011) with HA1 containing most antigen epitopes of HA (Caton et al., 1982). Swinepox virus (SPV) is the only member of the *Suipoxvirus* genus in the *Chordopoxvirinae* subfamily of the *Poxviridae*. Infection in nature is usually mild and occasionally causes localized skin lesions that heal naturally (van der Leek et al., 1994).

Currently available swine influenza (SI) vaccines are inactivated whole virus. Application of these vaccines reduces the severity of disease but does not provide consistent protection against infection. We have engineered the recombinant swinepox virus rSPV/H3-2A-H1 to co-express the HA1 proteins from SIV subtypes H1N1 and H3N2 (Xu et al., 2012c). In this study we have examined the detailed immune responses of rSPV/H3-2A-H1 in mice and evaluated its safety, immunogenicity and protective efficacy in pigs.

2. Materials and methods

2.1. Viruses and cells

Wild type swinepox virus (wtSPV, Kasza strain, ATCC VR-363) and PK-15 cells were purchased from the American Type Culture Collection. rSPV/H3-2A-H1 has been constructed and identified. A crude viral stock was prepared and rSPV/H3-2A-H1 and wtSPV titers were determined as described previously (moi = 0.1) (Lin et al., 2011). SIV H1N1 (A/swine/Shanghai/1/2005) and H3N2 (A/swine/Guangxi/1/2004) strains were provided by Dr. Xian Qi (Jiangsu provincial center for disease prevention and control) were propagated only once in specific

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pathogen-free embryonated eggs (Nanjing Veterinary Drug and Instrument Factory, Nanjing, China). MDCK cells were used for SIV isolation and titration assay. All experiments involving SIV were conducted using biosafety level 3 procedures.

2.2. Mouse model

Forty-five 7-week-old female BALB/c mice (the Animal Center of Nanjing Army Hospital, Nanjing, China) were randomly divided into three groups (15 mice per group). Groups 1 and 2 were inoculated with rSPV/H3-2A-H1 or wtSPV, respectively, at the same dose of $0.2 \times 10^{7.0}$ $TCID_{50}$ in 0.2 mL EMEM and applied over the four legs. Group 3 was inoculated with 0.2 mL EMEM. All inoculations were administered intramuscularly (IM) three times at 1, 21 and 35 days post inoculation (dpi). At 21, 35 and 42 dpi, five mice of each group were euthanized and sera samples were obtained for detection of the antibody against SIV. At the same time, the lymphocytes were isolated from the spleens for measurement of the SIV-specific T lymphocyte proliferation responses. Moreover, the supernatants of the lymphocytes stimulated with purified SIV HA1 antigen at 35 dpi were obtained for evaluation of the Th1-type cytokine INF-γ and Th2-type cytokine IL-4.

2.3. Swine experiment

Thirty-three Yorkshire and Landrace crossbred pigs (seronegative for SIV and SPV) were obtained from Kangle farm (Changzhou, China). The pigs were weaned at the age of 3 weeks, then delivered to the Animal Disease Research Center of Agriculture Institute of Jiangsu Province and allowed to acclimate to their new environment and new feed for two weeks. Thirty of them were randomly assigned to six groups (5 pigs per group). Three pigs were included as environmental controls (unvaccinated and unchallenged). Each group was housed separately in an individual specified pathogen-free isolation room. At 5 weeks of age, groups 1 and 2 were individually inoculated IM in the neck area with rSPV/H3-2A-H1 at $1 \times 10^{7.0}$ $TCID_{50}$ in 1 mL EMEM per pig, group 3 and 4 with wtSPV at $1 \times 10^{7.0}$ $TCID_{50}$ in 1 mL EMEM per pig, and groups 5 and 6 was inoculated with 1 mL EMEM per pig. Pigs were closely monitored daily within the first 7 day post-vaccination. At 21 dpi, sera were collected to detect neutralizing antibodies (NA) against SIV and the peripheral blood mononuclear cells (PBMC) were isolated to evaluate SIV-stimulated production of INF-γ and IL-4. Then groups 1, 3 and 5 all pigs were challenged with $2 \times 10^{5.0}$ $TCID_{50}$ H1N1 SIV, and groups 2, 4 and 6 all pigs were challenged

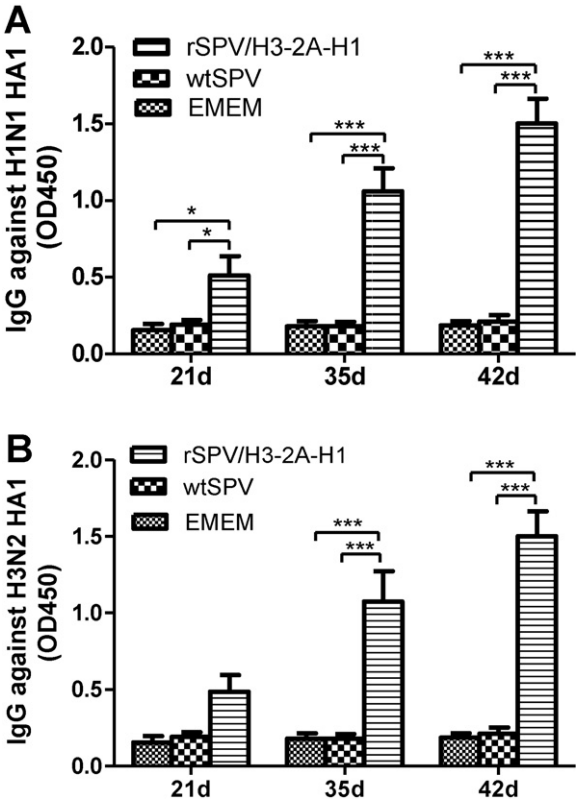

Fig. 1. IgG antibodies in mice induced by rSPV/H3-2A-H1 (a single sera dilution 1:100). Data were shown as mean ± SEM. (A) H1N1 SIV-specific IgG antibodies. (B) H3N2 SIV-specific IgG antibodies. $^{*}P < 0.05$ and $^{***}P < 0.001$.

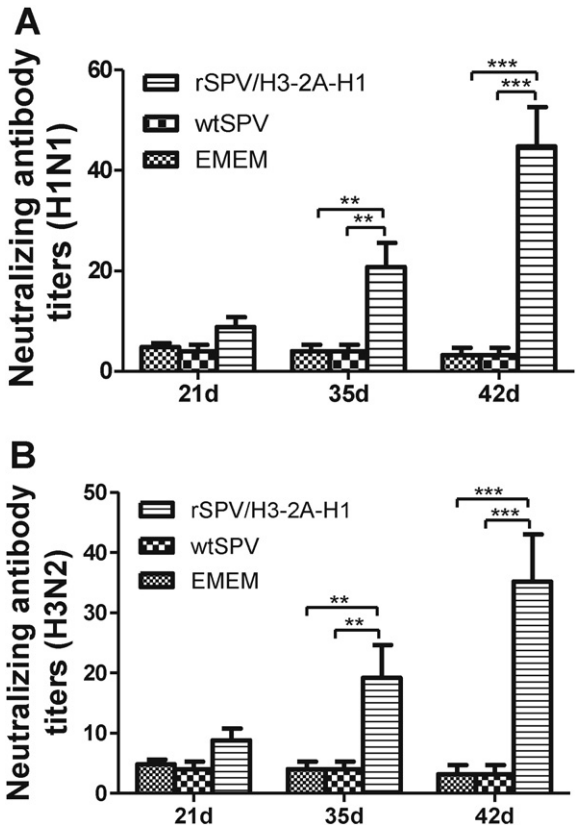

Fig. 2. rSPV/H3-2A-H1 induced SIV-specific neutralizing antibodies in mice. (A) H1N1 SIV-specific neutralizing antibodies. (B) H3N2 SIV-specific neutralizing antibodies. Data were shown as mean ± SEM. $^{**}P < 0.01$ and $^{***}P < 0.001$.